Paper No. 30

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte WILLIAM E. BENTLEY, and
RYAN T. GILL

Appeal No. 2003-0903
Application No. 09/534,366

ON BRIEF

Before SCHEINER, GRIMES, and GREEN, Administrative Patent Judges.

GRIMES, Administrative Patent Judge.

DECISION ON APPEAL

This is a decision on appeal under 35 U.S.C. § 134 from the examiner's final rejection of claims 1-32, all of the claims in the application. Claim 1 is representative and reads as follows:

1. A method for differential display analysis of mRNA via a single amplification, the method comprising the steps of:

adding a reverse transcription reaction first primer mixture comprising 13 random 10 and timer primers in equal molar amounts selected from the group consisting of RT1, RT2, RT3, RT4, RT5, RT6, RT7, RT8, RT9, RT10, PCR1, PCR3 and PCR5 to a first nucleic acid sample including a first mixture of mRNA to form a first primer/first nucleic acid sample mixture;

adding said first primer mixture to a second nucleic acid sample including a first mixture of mRNA to form a first primer/second nucleic acid sample mixture;

incubating said first primer/first nucleic acid sample mixture to produce a first population of cDNA;

incubating said first primer/second nucleic acid sample mixture to produce a second population of cDNA;

adding a PCR amplification reaction second primer mixture comprising 20 random 10 and I liner primers in equal molar amounts selected from the group consisting of RT1, RT2, RT3, RT4, RT5, RT6, RT7, RT8, RT9, RT10, PCR1, PCR2, PCR3, PCR4, PCR5, PCR6, PCR7, PCR8, PCR9, and PCR10 to said first population of cDNA to form a second primer/first population of cDNA mixture;

adding said second primer mixture to said second population of cDNA to form a second primer/second population of cDNA mixture;

amplifying said second primer/first population of cDNA mixture to produce a third population of cDNA;

amplifying said second primer/second population of cDNA mixture to produce a fourth population of cDNA;

identifying the presence or level of mRNA in said third population of cDNA, wherein the first mixture of mRNA was amplified in a single amplification; and

identifying the presence or level of mRNA in said fourth population of cDNA, wherein the second mixture of mRNA was amplified in a single amplification.

The examiner relies on the following references:

Liang et al. (Liang '672)	5,599,672	Feb. 4, 1997
Heyneker	6,057,100	May 2, 2000

Stratagene Catalog (Stratagene), "Gene Characterization Kits," p. 39 (1988)

Su et al. (Su), "Optimized Chemilumin-escent Detection of DNA Amplified in the Exponential Phase of PCR," BioTechniques, Vol. 17, No. 4, pp. 734-736 (1994)
Liang et al. (Liang 1995), "Analysis of Altered Gene Expression by Differential Display," Methods in Enzymology, Vol. 254, pp. 304-321 (1995)

Fislage et al. (Fislage), "Primer design for a prokaryotic differential display RT-PCR," Nucleic Acids Research, Vol. 25, No. 9, pp. 1830-1835 (1997)

Claims 1-32 stand rejected under 35 U.S.C. § 103. The examiner rejected most of the claims based on Liang '672 and Fislage; the remaining claims were rejected based on those references combined with one or more of Stratagene, Su, Heyneker, and Liang 1995.

We reverse.

Background

The specification discloses "methods and materials useful for performing reverse transcription-polymerase chain reaction [RTPCR] in prokaryotic cells." Page 1. In particular, the specification discloses methods for "differential display"; i.e., measuring differences in gene expression in response to specific stimuli.

The specification provides a useful synopsis of the state of the art. In the following passages, the disclosures attributed to Liang and Fislage are, for practical purposes, the same teachings that are relied on by the examiner. The specification states that

> differential display has not been widely utilized in prokaryotic systems due to a lack of polyadenylation at the 3' end of prokaryotic mRNA. The absence of polyadenylation prevents the initiation of cDNA by the 3'-anchored primers of the eukaryotic differential display method developed by Liang et al. However, an analogous system of differential display for prokaryotes was

> developed by Fislage et al. using non-anchored primers that would permit the initiation of cDNA in prokaryotes. . . .
>
> Through a detailed statistical evaluation of the coding regions extracted from bacterial genetic databases, Fislage et al. designed ten RT primers for the 3' end and ten PCR primers for the 5' end of prokaryotic mRNA. These primers have increased specificity in the 3' region and the 5' regions surrounding E. coli genes and decreased specificity for rRNA or other abundant small RNA species such that mRNA were preferentially transcribed. The RTPCR methodology of Fislage et al. used one RT primer in combination with a single PCR primer for an RTPCR reaction, which was subsequently repeated for each primer set so that 100 different amplifications were run for every sample. . . .

Pages 11-12.

The specification discloses an improvement on the Fislage method. The disclosed process uses a combination of Fislage's prokaryotic primers in order to "improve differential display analysis via one amplification, rather than requiring a series of amplifications." Page 12.[1] The specification discloses that "[a]fter a systematic analysis of several primer combinations and incorporation of several new primers, one combination appeared most effective for amplifying mRNA to a significant level." Page 21. That combination uses all ten of Fislage's RT primers plus three of Fislage's PCR primers in the reverse transcription reaction, and uses all twenty of Fislage's primers in the PCR reaction. See id.

Discussion

Claim 1 defines the basic method that is claimed. The method comprises sequential reverse transcription (RT) and PCR reactions, carried out in parallel

[1] The specification does not expressly concede that the primers used therein are the same as Fislage's, but the sequence of the primers (specification, page 19) appears to be the same as those disclosed by Fislage. Appellants have not disputed the examiner's contention that the specification's primers are the same as those of Fislage.

on two separate sample mixtures (i.e., a reference sample and an experimental sample). The reverse transcription reaction includes all ten of Fislage's RT primers together with three of Fislage's PCR primers (designated PCR1, PCR3, and PCR5 in the specification). The PCR reaction in the claimed method includes all ten RT primers and all ten PCR primers. Following PCR amplification, the "presence or level of mRNA" in the two samples is compared.

The examiner rejected the claims as obvious. All of the examiner's obviousness rejections depend on the combination of Liang '672 and Fislage, and therefore we can consider them together. The examiner characterized Liang '672 as teaching a differential display method. See the Examiner's Answer, pages 4-5. The examiner also noted that Liang teaches that more than one primer can be used in the RT and/or PCR reactions. See the Examiner's Answer, page 6. The examiner also quoted Liang's guidance that

> [t]he use of more than one of each primer will increase the number of mRNAs identified in each reaction and the total number of primers to be used will be determined based upon the desired method of separating the cDNAs such that it remains possible to fully isolate each individual cDNA.

Liang '672, column 7, lines 37-42.

The examiner conceded that Liang '672 does not teach the specific primers recited in the claims, and relied on Fislage to make up for that deficiency. He concluded that it would have been obvious

> to combine the differential display using primer mixture method of Liang with the primer group of Fislage used by Fislage in differential display since Fislage states "In general, primers were selected for a high frequency of occurrence within the coding genome of E. coli. Furthermore, a high GC content for the 10 mers and a lower GC

> content in the case of the 11mers was preferred . . .” Fislage further motivates the use of these primers in differential display by noting “We have developed a primer set for a prokaryotic differential display of mRNA in the Enterobacteriaceae group . . . “

Examiner’s Answer, pages 6-7.

Appellants argue, in a nutshell, that the cited references do not suggest the specific combinations of primers that are recited in the claims. See the Appeal Brief, pages 11-15. Thus, Appellants assert that the rejection is based on improper hindsight and should be reversed.

“The PTO has the burden under section 103 to establish a prima facie case of obviousness. It can satisfy this burden only by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings of the references.” In re Fine, 837 F.2d 1071, 1074, 5 USPQ2d 1596, 1598 (Fed. Cir. 1988) (citations omitted). An adequate showing of motivation to combine requires “evidence that ‘a skilled artisan, confronted with the same problems as the inventor and with no knowledge of the claimed invention, would select the elements from the cited prior art references for combination in the manner claimed.’” Ecolochem, Inc. v. Southern Calif. Edison Co., 227 F.3d 1361, 1375, 56 USPQ2d 1065, 1075 (Fed. Cir. 2000)

In this case, we agree with Appellants that the examiner has not adequately explained how the references would have suggested the particular combination of primers recited in the claims. As Appellants point out, even though Fislage discloses all of the primers recited in the claims, the primers are

used in the prior art in combinations of a single RT primer and a single PCR primer. The examiner has pointed to nothing in Fislage that would have suggested using more than one of each primer in a given reaction.

The only suggestion of multiple primers pointed to by the examiner is in Liang '672. That statement, however, falls short of the specificity required to support a prima facie case under § 103. All Liang '672 says is that more than one RT or PCR primer can be used in a given reaction; the reference then goes on to say that the number of primers should be low enough that all the individual cDNAs can be isolated. The examiner has not explained how this limited suggestion would have led those skilled in the art to a process using thirteen and twenty primers, respectively, in the RT and PCR reactions.

In addition, we find no explanation by the examiner of why it would have been obvious to include any of Fislage's PCR primers in the RT reaction. Fislage discloses that the 11mer RT primers were designed to hybridize in the 3' regions of bacterial RNA, while the 10mer PCR primers were designed to hybridize in the 5' regions of the bacterial RNA. See the abstract. That is, the PCR primers were designed to hybridize to the wrong end of the bacterial RNA to serve as primers for reverse transcription; using them for reverse transcription would have been expected to send the reverse transcript directly off the 5' end of the RNA. The examiner has provided no explanation of why it would have been obvious to include any of these primers in the RT reaction, when they would have been expected to be inoperative for reverse transcription.

Summary

The examiner has not adequately shown that Liang '672 and Fislage would have suggested the method of claim 1 to those of ordinary skill in the art. The other references cited by the examiner were relied on merely to meet limitations of the dependent claims. The other references therefore do not overcome the deficiency of the basic combination. All of the rejections are reversed.

REVERSED

Toni R. Scheiner	)
Administrative Patent Judge	)
	)
	)
	) BOARD OF PATENT
Eric Grimes	)
Administrative Patent Judge	) APPEALS AND
	)
	) INTERFERENCES
	)
Lora M. Green	)
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